General Protocol for Immunofluorescent Antibody Staining

1. Fixation
   4% parafomaldehyde
   0.1 M Phosphate Buffer (PB)
   4°C (on ice) for 1 hour

2. Wash 2 hours in PBS (change PBS a few times)

3. Put in 30% sucrose/0.1 M PB O/N or until embryos sink

4. Embed in OCT, freeze on dry ice, store in –80° freezer

5. Section on cryostat (7-10µm)

6. Wash slides in Wash Soln:
   1% Heat-inactivated goat or sheep serum
   0.1% Triton X-100
   in PBS
   1 X 10 min

7. Dilute Primary Ab in wash at appropriate concentration

8. Add around 150µl per slide in flat humidified chamber, leave O/N at 4°C (can cover with parafilm to avoid drying out, put wash in cold room)

9. Wash 3 X 20 min at RT (1st wash for 30 min if really cold)

10. Dilute Secondary Ab in wash (1:200 for anti-mouse Cy3)

11. Add 150µl per slide, leave 1 hour at RT in humidified chamber protected from light

12. Wash 2X 30 min at RT

13. Mount in 80% glycerol

14. Examine under scope and photograph within 2-3 days.
   Store at 4°C when not examining